Significance of Oral Glucose Tolerance Tests in Non-alcoholic Fatty Liver Disease Patients with a Fasting Plasma Glucose Level of <126 mg/dL and HbA1c Level of ≤6.4% in Japan


Abstract

Objective The aim of this study was to clarify the indications for oral glucose tolerance tests (OGTT) in non-alcoholic fatty liver disease (NAFLD) subjects with a HbA1c level of ≤6.4%, fasting plasma glucose (FPG) level of <126 mg/dL and no history of diabetes.

Patients A total of 569 NAFLD subjects underwent 75-g OGTT. The plasma glucose and insulin levels were analyzed periodically for three hours during the OGTT examinations. Impaired fasting glucose (IFG) was defined as a plasma glucose level of 100 mg/dL to <126 mg/dL. Diabetes was defined as a two-hour post-load plasma glucose level of ≥200 mg/dL. Elevated insulin resistance was defined as a homeostasis model assessment-insulin resistance (HOMA-IR) of ≥2.5. Insulin secretory insufficiency was defined as an insulinogenic index of <0.4.

Results The prevalence of diabetes on the OGTT was 7.7% (44/569) among the NAFLD patients with an HbA1c level of ≤6.4%, FPG level of <126 mg/dL and no history of diabetes. A multivariate analysis showed that diabetes occurred more frequently when the subjects had IFG [odds ratio (OR) 5.13; 95% confidential interval (CI) 3.01-8.76; p<0.001] and an HbA1c level of 5.7-6.4% (OR 5.45; 95% CI 3.33-8.93; p<0.001). Of the NAFLD subjects with both IFG and an HbA1c level of 5.7-6.4%, 22.8% (28/123) exhibited a pattern of diabetes on OGTT. Regarding insulin dynamics, among the NAFLD subjects with both IFG and an HbA1c level of 5.7-6.4%, 25.2% (31/123) had elevated IR alone, 25.2% (31/123) had insulin secretory deficiency alone and 27.6% (34/123) had both elevated insulin resistance and insulin secretory deficiency.

Conclusion NAFLD subjects with IFG and an HbA1c level of 5.7-6.4% should undergo OGTT in order to determine whether they have diabetes and/or abnormal insulin dynamics.

Key words: non-alcoholic fatty liver disease, oral glucose tolerance test, type 2 diabetes mellitus

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the more common causes of chronic liver disease in the Western world and many Asian nations (1-6). NAFLD is considered to be the liver component of metabolic syndrome (7-9) and is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, chronic kidney disease and type 2 diabetes (T2DM) (10-16). In addition, the presence of NAFLD is associated with a high risk of developing cardiovascular disease and stroke (17, 18). Hence, NAFLD is
emerging as a new significant health problem in many countries. A substantial problem is that NAFLD is strongly associated with T2DM. The coexistence of NAFLD and T2DM is clinically important for the following reasons: 1) T2DM is an independent predictor of hepatic fibrosis progression in patients with NAFLD (19) and 2) the presence of T2DM is pivotal with respect to increased risks of cardiovascular disorders and the development of hepatocellular carcinoma in the setting of NAFLD (20, 21). Therefore, early intervention to prevent or improve T2DM is required in order to obtain a good prognosis in NAFLD patients. The physicians in charge of NAFLD patients should thus detect T2DM in the early stage. According to population-based studies conducted in Asia, only 37% of diabetic patients fulfill both the fasting and two-hour plasma glucose criteria (22). Although oral glucose tolerance tests (OGTT) are adequate for making a strict diagnosis of T2DM, they are not routinely applied in NAFLD subjects due to the high cost and inconvenience.

Hence, it is an urgent issue to determine the indications for OGTT in NAFLD subjects. This topic must be addressed in studies with large numbers of NAFLD patients in whom the glucose and insulin levels are examined after oral glucose loading.

Against this background, we evaluated the prevalence of impaired glucose tolerance (IGT) and diabetes based on the findings of OGTT in Japanese subjects with NAFLD. In addition, we assessed the level of insulin secretion and degree of insulin resistance in the study participants. The strengths of the current study are the large sample size and comparison with control subjects exhibiting normal aminotransferase levels without fatty liver.

**Materials and Methods**

**Subjects**

A total of 823 Japanese subjects diagnosed with fatty liver on ultrasonography (23) and examined for oral glucose tolerance using 75-g glucose loading between January 1997 and December 2007 at the Department of Hepatology and Toranomon Hospital Health Management Center were enrolled in this study. Of the 823 subjects, 569 satisfied the following inclusion criteria: 1) a HbA1c national Glycohemoglobin Standardization Program (NGSP) equivalent value (%)) of ≤6.4% and fasting plasma glucose (FPG) level of <126 mg/dL; 2) no history of diabetes; 3) a current and past daily alcohol intake of <20 g/day; 4) negativity for hepatitis B surface antigens (HBsAg), anti-hepatitis C virus (HCV), antinuclear antibodies or antimitochondrial antibodies in the serum, as determined on a radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; 4) no underlying neoplasms or systemic disease, such as systemic lupus erythematosus or rheumatic arthritis; 5) the absence of malignancy on gastrofiberscopy, abdominal ultrasonography, chest X-ray and/or chest computed tomography; 6) levels of the tumor markers carcinoembryonic antigen, alpha-fetoprotein and prostate-specific antigen with the normal range. Subjects meeting the above criteria were enrolled regardless of whether their serum level of aminotransferase was within the normal range. Subjects meeting any of the following criteria were excluded from the study: secondary causes of steatohepatitis or drug-induced liver disease, alcoholic liver disease, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson’s disease and biliary obstruction.

All subjects underwent 75-g OGTT examinations after a 12-hour fast. The plasma glucose and insulin levels were analyzed before and 30, 60, 90, 120 and 180 minutes after oral glucose loading. Blood samples were obtained from all subjects six times during the OGTT after oral glucose loading. IGT was defined as a two-hour post-load plasma glucose level of 140-199 mg/dL. Diabetes was defined as a two-hour post-load plasma glucose level of 200 mg/dL (24). Impaired fasting glucose (IFG) was defined as a plasma glucose level of ≥100 mg/dL to <126 mg/dL. The index of insulin resistance was calculated for fasting glucose and insulin according to the homeostasis model for insulin resistance (HOMA-IR). Elevated insulin resistance (IR) was defined as a HOMA-IR of ≥2.5 (25). Insulin secretion was calculated according to the insulinogenic index (IGI), as follows: IGI = (Ins30-Ins0) / (Glc30-Glc0), Ins0: fasting plasma insulin (mU/L); Ins30: insulin 30 minutes after glucose intake (IU/mL); Glc0: FPG (mg/dL); and Glc30: plasma glucose 30 minutes after glucose intake (mg/dL). Insulin secretory insufficiency was defined as an IGI of <0.4 (26). The pattern of insulin dynamics was divided into the following four types based on the differences in HOMA-IR and IGI: 1) normal insulin dynamics, IGI≥2.5 and HOMA-IR<2.5; 2) insulin secretory insufficiency, IGI<0.4 and HOMA-IR<2.5; 3) elevated insulin resistance, IGI<0.4 and HOMA-IR≥2.5; 4) combination of insulin secretory insufficiency and insulin resistance, IGI<0.4 and HOMA-IR≥2.5. Fatty liver was diagnosed based on the presence of an ultrasonographic pattern consistent with a bright liver with stronger echoes in the hepatic parenchyma than in the renal or splenic parenchyma (23).

**Clinical and laboratory analysis**

The laboratory analysis was performed according to standard laboratory methods. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, USA). HBsAg was assessed using a radioimmunoassay (Abbott Laboratories, Detroit, USA). The serum biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, FPG, insulin and HbA1c. The serum insulin levels were measured with a solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA). The HbA1c level (%) was estimated as the NGSP equivalent
The results are presented as the mean ± standard deviation (SD) or as numbers with percentages. Statistical differences in quantitative data were determined using the Mann-Whitney U-test, Fisher’s exact probability test and Kruskal-Wallis test. Changes in the serum glucose and insulin levels between the NAFLD patients and control subjects during OGTT were analyzed using a one-way repeated measurement ANOVA. Significant predictors according to a univariate analysis were subsequently included in a forward, step-wise multiple logistic regression model in order to identify important predictive factors for the diabetes pattern on the OGTT. The Statistical Program for Social Sciences software package (SPSS 11.5 for Windows, SPSS, Chicago, USA) was used to perform all statistical analyses. A p value of <0.05 was considered to be statistically significant.

Results

Patient characteristics

Table 1 shows the characteristics on the day of the OGTT examinations in the NAFLD group. The mean age was 59.8 years, the mean BMI was 26.3, the mean FPG level was 95.5±7.5 mg/dL and the mean HbA1c level was 5.0±0.3%.

Prevalence and predictive factors of diabetes

According to the OGTT results, of the 569 NAFLD subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL, 198 (34.8%) were diagnosed as having IGT and 44 (7.7%) were diagnosed as having diabetes.

Table 2 shows the predictive factors for diabetes in the NAFLD subjects. The multivariate analysis showed that diabetes occurred more frequently among the NAFLD patients with a FPG level of 5.6-6.9 mg/dL [odds ratio (OR) 5.13; 95% confidential interval (CI) 3.01-8.76; p<0.001] and HbA1c level of 5.7-6.4% (OR: 5.45; 95% CI 3.33-8.93; p<0.001).

Fig. 1 shows the distribution of the normal, IGT and diabetes pattern on OGTT in each group classified based on the differences in FPG and HbA1c. Among the 123 subjects with both IFG and an HbA1c level of 5.7-6.4%, 28 (22.8%) were diagnosed as having diabetes on OGTT. In addition, 67 patients (54.5%) were diagnosed as having IGT.

Insulinogenic index and HOMA-IR based on the differences in FPG and HbA1c

Fig. 2 shows the prevalence of insulin secretory insufficiency and elevated insulin resistance in each group classified based on the differences in FPG and HbA1c. Among the 123 NAFLD subjects with both IFG and an HbA1c level of 5.7-6.4%, 31 (25.2%) exhibited elevated insulin resistance, 31 (25.2%) had insulin secretory deficiency and 34 (27.6%) displayed both elevated insulin resistance and insulin secretory deficiency.

Discussion

We herein described the state of glucose and insulin after OGTT in NAFLD Japanese subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL. The strengths of the present study include the following points: 1) the large number of subjects with NAFLD, 2) the evaluations of insulin resistance and insulin secretion in all enrolled patients.

The present study showed several important findings with regard to the prevalence of abnormal oral glucose tolerance in Japanese NAFLD subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL. First, approximately 40% of the NAFLD subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL showed abnormal glucose tolerance on OGTT. In particular, among the 123 subjects with both IFG and an HbA1c level of 5.7-6.4%, approximately one-fifth of the NAFLD patients were diagnosed as having diabetes on OGTT. Indeed, the HOMA-IR is also useful for screening diabetic patients, and if OGTT were not performed, diabetes cases may be missed. Wong et al. reported that T2DM and IGT cannot be accurately predicted by any fasting glucose cut-off values among Chinese NAFLD patients without a history of diabetes (27). The present investi-
Second, the multiple regression analysis showed FPG and HbA1c to be independent predictors of a diabetes pattern on OGTT in the NAFLD subjects. These results suggest that IFG and an HbA1c level of 5.7-6.4% are associated with an increased prevalence of a diabetes pattern on OGTT in NAFLD subjects. In fact, of the NAFLD subjects with an HbA1c level of 5.7-6.4% and IFG, approximately 23% showed a diabetes pattern on the OGTT examinations. Although OGTT has the disadvantages of greater cost and inconvenience, physicians in charge of NAFLD patients with IFG and an HbA1c level of 5.7-6.4% should perform OGTT in order to evaluate the presence of diabetes. Yun et al. reported that OGTT should be conducted in young men with

**Table 2. Predictive Factors for Diabetes Pattern in Oral Glucose Tolerance Test in NAFLD Patients**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>p</td>
</tr>
<tr>
<td>Age (per 10 years)</td>
<td>1.47 (1.17-1.84)</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender (male / female)</td>
<td>1.72 (1.07-2.76)</td>
<td>0.026</td>
</tr>
<tr>
<td>Body mass index (per 5)</td>
<td>1.64 (1.20-2.27)</td>
<td>0.002</td>
</tr>
<tr>
<td>AST (IU/L, ≥34/≤34)</td>
<td>0.97 (0.44-2.13)</td>
<td>0.932</td>
</tr>
<tr>
<td>ALT (IU/L, ≥42/≤42)</td>
<td>1.57 (0.82-3.03)</td>
<td>0.177</td>
</tr>
<tr>
<td>GGT (IU/L, ≥109/≤109)</td>
<td>1.17 (0.47-2.93)</td>
<td>0.746</td>
</tr>
<tr>
<td>Platelet (×10^9/mm^3, ≥20/＜20)</td>
<td>0.63 (0.23-1.74)</td>
<td>0.372</td>
</tr>
<tr>
<td>APRI (≥0.5/＜0.5)</td>
<td>0.67 (0.290-1.536)</td>
<td>0.342</td>
</tr>
<tr>
<td>Albumin (g/dL, ≥3.9/＜3.9)</td>
<td>1.07 (0.68-1.69)</td>
<td>0.762</td>
</tr>
<tr>
<td>Triglyceride (mg/dL, ≥150/＜150)</td>
<td>1.95 (1.24-3.08)</td>
<td>0.004</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL, ≥220/＜220)</td>
<td>1.15 (0.74-1.80)</td>
<td>0.533</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL, ≥40/＜40)</td>
<td>0.59 (0.34-1.03)</td>
<td>0.065</td>
</tr>
<tr>
<td>FPG (mg/dL, ≥100-＜126/＜100)</td>
<td>8.38 (5.03-13.96)</td>
<td>＜0.001</td>
</tr>
<tr>
<td>HbA1c (NGSP %, 5.7-6.4/＜5.6)</td>
<td>8.75 (5.47-13.98)</td>
<td>＜0.001</td>
</tr>
</tbody>
</table>

*ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidential Interval, DM: diabetes mellitus, FPG: fasting plasma glucose, GGT: gamma-glutamyltransferase, HbA1c: hemoglobin A1c, HDL: high density lipoprotein, OR: odds ratio

Figure 1. Oral glucose tolerance pattern based on the differences in the fasting plasma glucose and HbA1c levels among the NAFLD patients with an HbA1c level of ≤6.4%, FPG level of <126mg/dL and no history of diabetes.

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NAFLD who exhibit elevated liver enzymes and IFG in order to predict the risk of T2DM (28). In addition, Heianza et al. reported that the general population with IFG and an HbA1c level of 5.7-6.4% tends to have a greater risk of the onset of DM based on the findings of a large-scale follow-up study (29). Furthermore, the present results indicate that HbA1c and FPG are strong predictors for T2DM compared with liver enzymes. Although a recent study found a higher BMI and older age to be associated with impaired glucose metabolism in NAFLD patients (30), these values were not identified to be significant in the multivariate analysis in that study. We do not consider these factors to have had a sufficient impact on the rate of DM in this study.

Third, among the NAFLD patients with IFG and an HbA1c level of 5.7-6.4%, most demonstrated elevated insulin resistance and/or insulin secretory insufficiency; one-fourth had elevated insulin resistance, one-fourth had insulin secretory insufficiency and one-fourth had both elevated insulin resistance and insulin secretory insufficiency. Several other authors have reported that postprandial hyperinsulinemia is universal in non-diabetic patients with NAFLD (31-34). Therefore, it is recommended that NAFLD patients with IFG and an HbA1c level of 5.7-6.4% be examined using OGTT in order to evaluate whether they have elevated insulin resistance and/or insulin secretory insufficiency.

Recent studies have demonstrated that non-invasive serum markers can be used to predict the onset of DM (35-37). We previously estimated the aspartate aminotransferase platelet ratio index (APRI), calculated as the AST [ULN]/platelet count [10^3/mL] x 100, from a database (37). Unfortunately, our results did not show the APRI to be a predictor of DM, as most of our patients had either no or mild liver fibrosis.

The prevalence of NAFLD has increased dramatically in many nations, including Japan, over the past decades. At present, according to Japanese annual health check reports, 9-30% of Japanese adults demonstrate evidence of NAFLD on ultrasonography. Since approximately 10% of individuals with NAFLD have non-alcoholic steatohepatitis (NASH), the prevalence of NASH is estimated to be 1-3% of the adult Japanese population (21). It was also recently reported that T2DM may occur in NAFLD patients (15, 16) and that NAFLD subjects with T2DM have an increased risk of hepatocellular carcinoma (20, 21). Therefore, in subjects with NAFLD and T2DM, the management of DM is very important for improving the prognosis. The present findings indicate that OGTT is a useful test for detecting diabetes, insulin resistance and insulin secretory insufficiency in NAFLD subjects with IFG and an HbA1c level of 5.7-6.4%.

The present study is associated with several limitations. First, the NAFLD subjects did not undergo liver biopsies. In addition, although NAFLD can be categorized into simple steatosis or steatohepatitis, the present study was undertaken without histologically differentiating between simple steatosis and steatohepatitis. Second, control subjects without NAFLD were not evaluated. Third, our cohort comprised Japanese subjects only; this heterogeneity makes it difficult to interpret the results of our study.

In conclusion, NAFLD subjects with IFG and an HbA1c level of 5.7-6.4% should undergo OGTT in order to determine whether they have diabetes, elevated insulin resistance and/or insulin secretory insufficiency.

The authors state that they have no Conflict of Interest (COI).
Acknowledgement

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